THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

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Dear Joshua:

Since last I wrote you the roof has sort of fallen in on my problem but I believed has allowed for a thorough clarification of the issues.

I've enclosed a summary of my thinking and some of the evidence for it. I want to apologize for its difficulty but I believe I am hanging on to a literary monster.

I've sent this to you for several reasons:

1. You seemed interested
2. I would like your opinion of the theory
3. Are the facts sufficient or how far do I have to go with those exponentially increasing factors (see text)
4. Can you help with the symbology or is it adequate
5. What to do with the already defined terms , prophage, lysogenic and non-lysogenic cells, host-induced modification and immunity
6. Can this be made intelligible to other than a restricted audience?

Best regards

Sincerely

Norton

Theory

With respect to the manifestations of lysogeny by the A phages of Salmonella, the following is proposed.

All strains capable of adsorbing the phage contain within their genetic complement material which is homologous to that of the phage. Productive (phage proliferation either through a lytic or lysogenizing cycle) or non-productive responses are primarily manifestations of the allelic configurations of a number of loci in the host and the phage.

A temperate bacteriophage (non-virulent mutant) can come in contact with a variety of cell types.

1. Cells which either spontaneously or following induction produce precisely the infecting phage and abort the infection. This represents what we may call homologous immunity.

2. Cells which do not produce the homologous phage but still as a primary response abort the infection. The productive responses often produce host-modified phage. This may be called heterologous immunity.

3. Cells for which there is no evidence of homologous phage production and within which phage can readily undergo a productive response. These are the typical sensitive, non-lysogenic strains.

With virulent mutants of temperate phage all of the above cell types are met but all productive responses are via a lytic cycle.

The major point for consideration is that the immunity or lack thereof is not because of the presence or absence at a particular site of a prophage but rather is a phenotypic response brought about by the interaction of appropriate phage and bacterial alleles. With homologous immunity there is no possible recombination between the phage and prophage to give anything different and thus essential the completeness of the immunity. With heterologous immunity the different alleles in phage and prophage can recombine to give rise to phage of changed plating

efficiency (host-induced-modification). In this light virulent mutants of temperate phages may be viewed as alleles at what we might call compatability? loci which can not with any known set of of bacterial alleles produce the immunity requisite for lysogenization (transformation of the bacterial chromosome?) to occur.

Experimental

The three components of the system used are S. typhimurium strain LT2 (strain A), S. typhimurium var. Copenhagen and phage P22. The following linkage map for P22 can be drawn on the basis of phage by phage crosses in strain A. The mutant symbols have the following meaning and can be readily scored on nutrient agar; t turbid halo, v_2 virulent, y yellowish halo, l large halo, v_1 semi-virulent, m modifies expression of l and y.

I II

There are as seen two linkage groups. Phage by phage crosses give about 20% recombination between the two indicating as a maximum one round of mating. In order to follow the linkage groups we will arbitrarily place on them identifying markers for each of the components of the system according the hypothesis presented

	I	II
strain A	A	A'
strain B	B	B'
P22	C	C'

We will also call P22 as given P1 (= C,C').

P1 on strain A Strain A acts as a typical sensitive host for P1. It is readily lysogenized by P1 temperate exhibiting the multiplicity effect. All of the phage mutants breed true on A as far as can be tested.

P1 on strain B When P1 infects B about 20% of the input phage produces plaques on B. These plaques contain a variety of things. They contain P1 and also plaque morphology

mutants of P1. The mutants are produced in such a way as to define a particular phage allele at each locus of strain B. However the mutants as can be shown by single burst experiments are not equally frequent and are biased from one point in linkage group one . More frequent away from v_2. Since the platings are done on strain A we therfore define a marker B on linkage group one of stsain B which does not allow plating on A. Linkage group two assorts more or less at random. However phage with linkage group one from P1 and linkage group 2 from B have a different plating efficiency. This also depends in part as to wheh whether they have the v_2 allele of P1 or that of strain B.

There is also in an plaque of P1 on B a phage which plates well on B and poorly on A. It can not be found in the single burst experiment for obvious reasons. We have tabulated below the plating efficiencies of the various things derived from P1 on B making use of two other strains in addition to A and B whose derivation will be desbribed shortly.

	E.O.P.			
	A	B	C	D
P1	1	.2	.6	.6
P1 mutant linkage I	1	.2	.6	.6
P2 " " II	1	10^{-3}	.3	.3
P3 v_2 from B mutant II	1	"	.8	.8
don't have v_2 from B without linkage II from B yet				
P4	10^{-3}	1	.4	.4

P4 plaques are impossible to define with respect to other markers. Its genotype will be clarified below.

Strain B

In ormder to explicitly demonstrate that the phenomena described above can be ascribed to recombinational rather than mutational events the following series of experiments were accomplished. Strain B was lysogenized with P1 temperate.

By irradiating it with UV it was possible to obtain a"delysogenized" cell (strain C). Surprisingly this strain had lost its mutational capacities for P1 and plated it well. However it was found to be sensitive to a phage produced by strain B (P5). P5 contained the postulated phage alleles in strain B as shown by appropriate crosses. It contains the postulated locus B linked to v_2 which does not allow it to plate on A explaining the bias in the mutant output. Among the recombinants of P1 by P5 are the expected classes but with much reduced frequency as compared to P1 by B crosses (an interesting point about phage recombination) Also P4 was produced . This turns out to have linkage group I of P5 and linkage group II of P1. The particular factor involved is more closely lnked to v_1 than m. Diagrammed below is the linkage group associated with (compatability factors) of the various phages . It should be first mentioned that P5 plates on strain C , not at all on B and like P4 on A.

P1	B	C'
P2	C	B'
P3	BC	B'
P4	B	C'
P5	B	B'

Since it was possible to delysogenize B after it was lysogenized with P1 an attempt was made to do this directly. Strain D was obtained in this manner. Thus we have two strains made sensitive in the laboratory which were different in the genetic composition of the phage which they had carried.

P4 and P5 Both P4 and P5 plate poorly on strain A . of the yield obtained with P5 on A 99% is the input and 1% something new, P6. Similarly with P4 we make P7

E.O.P

	A	B 3	C	D
P6	1	10	.01	.1
P7	1	1		

By appropriate crosses it can be shown that P6 is A B' or perhaps AB B'. When grown on strain C it gives P6, P6 mutants and a phage P8 which has picked up

is not unlike P2 C B'. When P6 is grown on strain D we again get P6 and ~~its~~ mutants (differentiates), and also P9 which appears to be identical with P5 B,B'. Thus ~~in~~ delysogenized cells remember what they had been lysogenized with and the mutation induced that delysogenized them was probably in linkage group II. It is quite conceivable that they can be delysogenized again, that is, induce a new mutation at the compatability locus and become sensitive to the phage they are now carrying.

Among the yield of P4 on strain A is P7 which has the following composition A C' or AB C'. With P1 on strains A and B we can therefore define its entire host modification system. P1 on B to P4 on A to P7 on B to P4 etc. (8) Thus we have achieved a closed system with turn over of linkage group I each time.

Miscellany

We have not yet obtained all of the combinations possible and investigated all of the possible cell and phage types with just the original three components of the system. It might well be that the series is infinite. Subtle differences are difficult to pick up and the neutrality of the other markers in all this remains to be shown. However it does seem clear that the compatability loci are linked to the V loci affecting lysogenizing ability. The alleles at the v_2 loci certainly have some effect (see P3) in fact the v_2 loci carried latently by the different cells all look slightly different and although in phage by phage crosses don't recombine there is some evidence that they will do so in phage by bacterial crosses where recombinant frequencies are much higher. We may cite for example a strain B cell which when treated with P1 temperate became heterogenotic for the psophage, that is, either spontaneously or by induction (much more efficiently induced than the usual lysogenic) produces P1, P5 and P2 and P4. The latter two amount to 30% of the yield while a cross of P1 and P5 give only a few percent of these. The lysates also contain of the heterogenote virulent phage.